A Gut Feeling About Seizures

The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet.

Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. *Cell* 2018;173:P1728–P1741.e13.

The ketogenic diet (KD) is used to treat refractory epilepsy, but the mechanisms underlying its neuroprotective effects remain unclear. Here, we show that the gut microbiota is altered by the KD and required for protection against acute electrically induced seizures and spontaneous tonic-clonic seizures in two mouse models. Mice treated with antibiotics or reared germ free are resistant to KD-mediated seizure protection. Enrichment of, and gnotobiotic co-colonization with, KD-associated *Akkermansia* and *Parabacteroides* restores seizure protection. Moreover, transplantation of the KD gut microbiota and treatment with *Akkermansia* and *Parabacteroides* each confer seizure protection to mice fed a control diet. Alterations in colonic lumenal, serum, and hippocampal metabolomic profiles correlate with seizure protection, including reductions in systemic gamma-glutamylated amino acids and elevated hippocampal GABA/glutamate levels. Bacterial cross-feeding decreases gamma-glutamyltranspeptidase activity, and inhibiting gamma-glutamylation promotes seizure protection *in vivo*. Overall, this study reveals that the gut microbiota modulates host metabolism and seizure susceptibility in mice.

Commentary

Drug-resistant epilepsy is a clinical challenge for 30% of patients with epilepsy and minimal therapeutic options exist (1). With the advent of new technologies and high-efficiency antiepileptic drugs (AEDs) still failing to effectively treat some epilepsy patients, there is a clear need for new therapeutic approaches (2). Until now, most causal changes associated with neurologic disorders have been isolated to abnormal brain function; however, mounting research suggest a role for the gut microbiota and gut–brain interactions in neurologic diseases, including epilepsy.

The low-carbohydrate, high-fat ketogenic diet (KD) was developed as a viable therapy for treatment of epilepsy since the 1920s (3). Results from clinical studies have shown that the KD is highly effective in treating drug-resistant epilepsy, with some of pediatric patients experiencing more than 50% reduction in seizure frequency (4). Although many variants exist to increase palatability, the classic KD is composed of a macronutrient ratio of 4:1 (4 g of fat to every 1 g of protein plus carbohydrates combined) (3). This regimen induces ketone body production in the liver through fat metabolism. Prior studies suggest that several pathways are affected by changes in ketone bodies, including improved mitochondrial function and genomics effects via alteration in chromatin modifiers, such as lysine deacetylases (5). However, how these changes decrease seizures remains elusive.

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After almost a century of being recognized as a treatment for drug-resistant epilepsy, a recent study by Olson et al. has identified a key mechanism by which the KD works to control seizures. The meticulously designed study by Olson and colleagues outlines the process by which the KD decreases seizures, specifically by modulating key bacterial species, which increase GABA/glutamate ratio levels to decrease seizures. They performed a combination of techniques to provide evidence that the KD alters the gut microbiome to decrease seizure activity. First, the authors used the highly drug resistant 6-Hz–induced seizure model, to demonstrate the involvement of the KD in seizure protection. The authors report that the KD reduced seizure activity by raising seizure thresholds and altering the gut microbiota.

Evidence supporting the antiseizure effects of the KD being mediated by the gut microbiota came from experiments where Olson et al. used animals that were reared germ free (GF) or treated with antibiotics. The KD had no effects on seizure threshold in the GF or antibiotic-treated mice, confirming that the gut microbiota is required for the KD-induced antiseizure effects. To cement the importance of the gut microbiota to seizure control, they showed that if GF pups were reared with specific pathogen-free (SPF) gut microbiota, the seizure protection was restored. Together, these findings suggest that the antiseizure effect of the KD is due to changes in the gut microbiota.

Using 16S rDNA sequencing, they next investigated whether specific microbes played a role in the antiseizure effects of the KD. Although some bacterial species were lost in response to the KD, the authors did find that *Akkermansia muciniphila (A. muciniphila)*, *Parabacterioides*, *Sutterella*, and *Erysipelotrichace-*

ae increased. Interestingly, prior work by Newell et al. (6) used the BTBR(T + tf/j) mouse model of autism spectrum disorder (ASD) and reported KD-induced changes in the gut microbiota that counteracted the ASD phenotype. The use of seizure models by Olson et al. demonstrated that the KD induced changes in the gut microbiota to decrease seizures.

Next, they tested the specific contribution of microbes to the antiseizure effects of the KD. Two species that were significantly enriched in KD-fed mice, *A. muciniphila* and *Parabacterioides*, were administered to antibiotic-treated animals. Intriguingly, intestinal enrichment and antiseizure effects occurred only when both species were given together. Bacterial reconstitution of *A. muciniphila* and *Parabacterioides* in GF mice also produced protective effects, further validating that the presence of these two bacteria was direct. Collectively, these findings are striking and identify for the first time a pivotal role of these specific species in seizure protection.

The initial studies used the drug-resistant 6-Hz–induced seizure model to demonstrate the antiseizure effects of the KD. This raised the question of whether the antiseizure effects of the KD on the gut microbiota were similar in other seizure models. The authors used the *Kcna1*$^{-/-}$ model of temporal lobe epilepsy, which develops spontaneous seizures and was previously reported to be reduced by the KD (7, 8). Similar to the drug resistant 6-Hz model, the KD increased *A. muciniphila* and *Parabacterioide* in *Kcna1*$^{-/-}$ mice although not to the same level. There were corresponding decreases in the incident and duration of seizures in response to the KD in the *Kcna1*$^{-/-}$ model. This is an important finding as it validates that the effect of the KD on specific microbial changes to decrease seizures expands to other seizure models.

To delve deeper into the mechanism of the antiseizure protection induced by gut bacteria, Olson et al. explored the metabolic profiles of KD treated mice and *A. muciniphila*- and *Parabacterioides*-enriched mice. Among their findings were a decrease in ketogenic gamma-glutamylated (GG) ketogenic amino acids and a reduction in glutamyltranspeptidase (GGT) activity, which are involved in maintaining amino acid levels (9). To determine if GG of amino acids was involved in seizure protection, they treated control animals with a GGT inhibitor, GGs Top, and reported increase in seizure thresholds similar to KD-treated control mice and a decrease in seizure activity in control fed *Kcna1*$^{-/-}$ mice treated with GGs Top. Moreover, when KD-fed *A. muciniphila*- and *Parabacterioides*-enriched mice were administered ketogenic amino acids, there was a decrease in seizure threshold and *A. muciniphila*- and *Parabacterioides*-enriched mice had reduced GGT activity. Altogether the authors are the first to report that the KD seizure protection is induced by selective enrichment of gut bacteria and provide a detail mechanism that involves specific microbial interactions that reduces bacterial GGT activity and cause a decrease in GG-amino acids that affects the GABA/glutamate ratios in the hippocampus, a brain region that is involved in seizures.

In sum, this is a well-crafted study that reveals important therapeutic strategies for treating and reducing seizures in epilepsy. However, there are several questions that remain unanswered. For example, it is unclear how amino acid changes in the gut affect GABA/glutamate levels specifically in the hippocampus. While unlikely, another provocative question raised from this study is whether modulation of GABA/glutamate levels is the main mechanism by which KD exerts its effect. Additionally, although reduced GGT activity led to changes in GABA/glutamate ratio, further studies are needed to show how alterations in GABA/glutamate levels are caused by KD therapy. Regardless of the remaining questions, there is clear gut–brain mechanisms in the anti-seizure effects of the KD, which inspires future epilepsy therapies that may target the gut microbiota directly.

by Farah D. Lubin, PhD, and Susan L. Campbell, PhD

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